UNITED STATES PATENT APPLICATION

for

SEMICONDUCTOR ELECTROCHEMICAL BIO-SENSOR ARRAY

Inventor:

Arjang Hassibi

prepared by:

BLAKELY, SOKOLOFF, TAYLOR & ZAFMAN LLP
12400 Wilshire Boulevard
Los Angeles, CA 90025-1026
(303) 740-1980

EXPRESS MAIL CERTIFICATE OF MAILING

"Express Mail" mailing label number: EV306655217US
Date of Deposit: February 10, 2004
I hereby certify that I am causing this paper or fee to be deposited with the United States Postal Service "Express Mail Post Office to Addressee" service on the date indicated above and that this paper or fee has been addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450
Leah Schwenke
(Typed or printed name of person mailing paper or fee)
Leah Schwenke
(Signature of person mailing paper or fee)
February 10, 2004
(Date signed)

SEMICONDUCTOR ELECTROCHEMICAL BIO-SENSOR ARRAY

[0001] This is a non provisional application based on the provisional application serial number 60/447,087 filed on February 13, 2003 and claims priority thereof.

COPYRIGHT NOTICE

FIELD OF THE INVENTION

[0003] The present invention relates to the field of chemical and biological analysis and synthesis.

BACKGROUND

[0004] There are many applications where one desires to detect and/or characterize a molecular motion and/or interaction (e.g., the occurrence of a binding event (either covalent or non-covalent) between two or more molecules, in a sample). Such applications find use in a variety of different fields, including

research and medical (e.g., diagnostic fields). Because of the importance of such applications to such a wide variety of different disciplines, an enormous variety of different protocols and methodologies have been developed to perform such applications.

[0005] Many protocols and methodologies use detectable labels, where labels can adversely modify the characteristics of a molecule(s) of interest. Some other protocols use indirect chemical or physical measurements to analyze the sample, yet in most of these protocols, either expensive and complicated detection devices must be employed, or sensitivity is quite limited.

[0006] As such, there is continued interest in the development of new methodologies for use in the characterization of molecular motion and/or interactions in a sample.

SUMMARY

[0007] According to one embodiment, a semiconductor electrochemical biosensor array (SEBA) is described. The SEBA includes an array of electrodes to receive sample material from an external source and sensor circuitry coupled to the array of electrodes. The sensor circuitry includes a plurality of sensor cells to analyze the sample material received at the array of electrodes.

[0008] In a further embodiment, the SEBA includes a decoder and control circuitry coupled to the sensor circuitry. The decoder selects which of the plurality of sensor cells are to be used to analyze the sample material. The control circuitry enables a user to activate a combination of electrodes and sensor cells. Further, the SEBA includes a function generator coupled to the control circuitry to generate signals for measurements, and reference elements coupled to the sensor circuitry.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The present invention will be understood more fully from the detailed description given below and from the accompanying drawings of various embodiments of the invention, which, however, should not be taken to limit the invention to the specific embodiments, but are for explanation and understanding only.

[0010] Figure 1 illustrates one embodiment of a semiconductor electrochemical biosensor array;

[0011] Figure 2 illustrates another embodiment of a semiconductor electrochemical biosensor array;

[0012] Figure 3 illustrates a block diagram of one embodiment of a semiconductor electrochemical biosensor array;

[0013] Figure 4 illustrates one embodiment of a triple electrode design;

[0014] Figure 5 illustrates one embodiment of a semiconductor electrochemical biosensor cell design;

[0015] Figure 6 illustrates one embodiment of a Charge Perturbation Signature (CPS) sensor circuit;

[0016] Figure 7 illustrates one embodiment of a differential Impedance Spectroscopy sensor circuit;

[0017] Figure 8 illustrates one embodiment of a single ended Impedance Spectroscopy sensor circuit with external load impedance;

[0018] Figure 9 illustrates one embodiment of a single ended Impedance Spectroscopy sensor circuit with no external load impedance;

[0019] Figure 10 illustrates one embodiment of an active electrode array circuit with three controllable voltage sources;

[0020] Figure 11 illustrate one embodiment of a binding experiment for an unknown species carried out with Impedance Spectroscopy (IS) analysis; and

[0021] Figure 12 illustrates one embodiment of an interactive test for CV analysis.

DETAILED DESCRIPTION

[0022] According to one embodiment, a semiconductor electrochemical biosensor array is described. The electrochemical biosensor array is a multiplexed, semiconductor based, electrochemical sensor array for chemical and biological analysis and synthesis. In one embodiment, a matrix of electrodes, configured to receive biological or chemical samples of interest, is connected to an integrated set of analysis and synthesis circuitry embedded in a semiconductor chip.

[0023] In the platform, integrated control circuitry activates independent cells within the array, and further analyzes or synthesizes are performed on bio-molecular samples on different sites. The sensor cells can perform various analyses methods, such as Charge Perturbation Signature (CPS) analysis, single-ended and differential Impedance Spectroscopy (IS), single-ended and differential Cyclic Voltammetry (CV), and also potentiometric measurements.

[0024] In a further embodiment, the array can also be used to work as an active electrode matrix, by putting particular potential on different electrode sets to activate electrophoretic experiments and amperometric processes.

[0025] In the following description, numerous details are set forth. It will be apparent, however, to one skilled in the art, that the present invention may be practiced without these specific details. In other instances, well-known

structures and devices are shown in block diagram form, rather than in detail, in order to avoid obscuring the present invention.

[0026] Reference in the specification to "one embodiment" or "an embodiment" means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment of the invention. The appearances of the phrase "in one embodiment" in various places in the specification are not necessarily all referring to the same embodiment.

[0027] **Figure 1** illustrates one embodiment of a semiconductor electrochemical biosensor array (SEBA) 100. SEBA 100 includes an electrode array 110 mounted on a substrate 120, and connectors 130 that connect the array 120 to a pad matrix 140 mounted on a semiconductor 150. Electrode array 110 serves as an input to pad matrix 140. According to one embodiment, electrode array 110 comprises an array of identical triple pad configurations that receive biological or chemical samples of interest from an external source.

[0028] Substrate 120 is a substance (organic or inorganic) that functions as a base layer. In one embodiment, substrate 120 is comprised of silicon oxide (SiO_2), while the electrode is comprised of gold (AU). In other embodiments, substrate 120 may be comprised of silicon nitride (N_4Si_3), and the electrode is comprised of aluminum (Al).

[0029] In a further embodiment, each electrode in array 110 is covered with a coating containing an enzyme, which causes the reaction of substrate 120 to produce a species to which the electrode responds. In particular, substrate 120 operates as an electron shuttle to transport electrons adsorbed from electrodes 110 to pad matrix 140 via connectors 130.

[0030] Pad matrix 140 is electrically coupled to connectors 130, as described above. Pad matrix 140 includes a matrix of sensor cells mounted on a semiconductor 150 substrate. The sensors of matrix 140 analyze biological and/or chemical samples received at electrode array 110. According to one embodiment, semiconductor 150 is a CMOS chip. However, one of ordinary skill in the art will appreciate that other semiconductor fabrication technologies may be implemented without departing from the scope of the invention.

[0031] **Figure 2** illustrates another embodiment of SEBA 100. In this embodiment, electrode array 110 is mounted directly on pad matrix 140. Moreover, pad matrix 140 is mounted semiconductor 150. Thus, in this embodiment, additional connectors are not implemented and the electrodes are directly connected to the internal sensors through the metal layers of the semiconductor processes. SEBA 100 also includes leads 170. Leads are used to transmit the analog or digital electrical signal, detected and/or amplified by the sensor circuitry, to the external devices. The transmitted signal subsequently can

be analyzed and correlated to the intrinsic characteristics of the samples within the electrode matrix.

[0032] **Figure 3** illustrates a block diagram of one embodiment of SEBA 100. Included in SEBA 100 is sensor circuitry 310 coupled to electrode array 110, decoder 320, reference elements 330, analysis control 340 and function generator 350. Sensor circuitry 310 is logic circuitry associated with sensor cells of pad matrix 140. Sensor circuitry 310 analyzes signals received from electrode array 110. In one embodiment, the output of sensor circuitry 310 is an analog signal that is transmitted to a central processing unit (CPU) 370 via an analog to digital (A/D) converter 360.

[0033] Decoder 320 is used to select various sensor cells to use for analysis. Reference elements 330 is a reference element bank that is implemented for the accurate loading of circuit elements. In one embodiment, reference elements 330 include tuning circuits (e.g. crystal), resistors and passive elements. Analysis control 340 enables a user of SEBA 100 to activate independent, or a combination of, sensors and electrodes, through user-defined inputs via sensor circuitry 310.

[0034] Function generator 350 is controlled by a SEBA 100 user to generate signals for active measurements, such as IS and CV analyses. In one embodiment, the function generator is capable of producing electrical waveforms with controllable amplitude and shape (e.g. sine-wave vs. square-wave) within a

particular frequency region. Function generator 350 may be internal or external to SEBA 100.

[0035] According to one embodiment, the inputs of the SEBA 100 platform are the addresses of a specific sensor cell or cells to be activated, and the analysis method type defined by the user. The output of the system is an analog output signal of the requested sensor cell or cells, based on the user-defined analysis method. For instance, in CPS analysis, the output signal is a transient signal generated by specific bindings of ions to the electrode-electrolyte interface of the active electrode. In the case of IS analysis, the output is a single tone with fixed amplitude and phase, in the case of CV analysis, a periodic waveform.

[0036] In one embodiment, signals obtained from electrode array 110 can be read sequentially. Further, the total analysis time and sampling rate for the entire matrix may also be calculated depending on the time assigned for analysis of each electrode of interest. Further, the analog signal generated from experiments may also be quantized by an analog to digital converter (A/D), which may be either internal or external to SEBA 100.

[0037] As described above, electrode array 110 comprises an array of identical triple pad configurations. **Figure 4** illustrates one embodiment of a triple electrode design. The configuration includes a Common electrode (C), an Active electrode (A), and a Reference electrode (R). The circuit also includes series

resistors R_1, R_{12}, and R_2, and capacitors C_1, C_{12}, and C_2. Each resistor, capacitor pair may be denoted as impedances (e.g., Z_1, Z_{12}, and Z_2,).

[0038] According to one embodiment, biological samples of interest are either spotted or chemically synthesized onto the Active electrode. The Reference electrode, positioned in close proximity to the Active electrode, is free of any target sample. The Common electrode is used in active measurements and the induced signal is introduced into solution via this electrode. The Common electrode can also be shared within the matrix.

[0039] In one embodiment, each triple electrode configuration is electrically connected to a single sensor cell in pad matrix 140. In a further embodiment, many possible topologies and sizes of the electrodes/pads may be configured to suit the application, depending on the application. For example, circular, rectangular and rectangular with a shared common electrode configurations may be implemented.

[0040] **Figure 5** illustrates one embodiment of a cell 500 of pad matrix 140. Cell 500 includes amplifier 510 and control register 550. In addition, cell 500 includes addressing lines and switches $S_0 - S_7$. Amplifier 510 is a differential amplifier that has inputs coupled to electrode A and electrode R, via switch S_3. The output of amplifier 510 is coupled to switch S_7.

[0041] In one embodiment, amplifier 510 provides a variable gain depending upon a specific application. Thus, amplifier 510 receives select bits S_8 and S_9 that are used to select which transistors within amplifier 510 are activated in order to choose a particular gain level. In a further embodiment, cell 500 is activated by two address lines (row-select and column-select) received from decoder 220.

[0042] Control register 550 within each cell 500 controls the internal functional switches S_0 - S_7 of the cell. Further, control register 550 can be programmed by the control-bus. Also, analog input and output lines are shared between all cells 500 in matrix 140. Depending on the application, the analog input and output lines can be routed from or into one node within the cell 500.

[0043] As described above, multiple electrochemical tests can be performed in the SEBA 100 platform. In one embodiment, the methods observe a certain macroscopic characteristic of the electrolyte-electrode interface region. Since a biochemical species of interest or target is located in this region, the spatial electrical characteristics obtained contain information about their structure, quantity, and behavior.

[0044] The electrochemical analysis techniques can be categorized into two distinctive categories. The first category is to measure the response of the electrolyte-electrode system. This is achieved by applying a perturbing signal into the system, such as a potential waveform. The methods which implement

the above methodologies are called active measurements (e.g., IS and CV analyses methods). The second analysis technique is known as passive measurement, wherein the electrical output of the system without perturbing the system is observed (e.g., CPS and various potentiometric measurement methodologies).

CPS and Potential Measurements

[0045] CPS is a Passive electrochemical technique to analyze DNA and other nano-biological entities. CPS technology is based on measuring the variation of the net charge of molecules (e.g., DNA) in proximity of an electrode when exposed to different reagents.

[0046] In the case of DNA analysis for example, primed single strand DNA molecules are immobilized on an electrode and placed in a solution containing polymerase. When nucleotides are added and extension occurs, the electrostatic response of a group of identical DNA molecules creates a unique waveform from which one can potentially use to recognize the pattern (for SNP or DNA sequencing), evaluate the mass (for gene expression).

[0047] **Figure 6** illustrates one embodiment of a cell 500 configured for CPS analyses. In the CPS analysis method, switches are turned on according to the adequate configuration for CPS analysis. In one embodiment, the switch

configuration is such that switches S_0, S_1, S_2 and S_4 are off while switches S_3, S_5, S_6 and S_7 are on. In addition, select bits S_8 and S_9 are controlled by the user to control the gain of amplifier 510.

[0048] For the CPS analysis method, a single low noise amplifier is needed. In one embodiment, the signal generated by CPS, has less than 10kHz bandwidth and is in the region of about 1kHz. As shown in **Figure 6**, the solution and samples on the electrodes are capacitively coupled to the sensor by the natural double-layer capacitance of the electrode-electrolyte interface

[0049] The binding phenomenon that occurs within the electrode cell creates a potential which is modeled by V(t), and the whole signal is amplified before being placed on the output line. In one embodiment, load elements can be added to the amplifier input to enhance the performance, depending on the application required.

IS (Differential and Single Ended)

[0050] Impedance spectroscopy (IS) is an active measurement where the electrode system is perturbed by an alternating signal of small magnitude. As a result, the way in which the system follows the perturbation in steady state is observed. In one embodiment, the induced signal is created by a voltage source with frequency ω and a relatively small amplitude (small signal with no nonlinear effects). The measured variable is the overall impedance, which the system has in frequency ω.

[0051] The impedance measured is a function of three factors: mobility of ions in the solution and their diffusion rate; the surface electrical characteristics of the interface; and the size and shape of both a reservoir and the electrodes. Because the observed impedance is a function of the system, any structural or quantitative change which electrically affects the system in steady state can be observed and measured.

[0052] **Figure 11** illustrates one embodiment of a binding experiment for unknown species (species "X") carried out with Impedance Spectroscopy (IS) analysis. The surface characteristic of the electrode system (the surface double layer capacitance) is different in the case of binding, (e.g., when "A" is truly present at the surface) compared to the non-binding effect (e.g., when species "A" does not find its complimentary). IS analysis can potentially differentiate

between the above two scenarios, and thus detect the existence of species "A" on an electrode.

[0053] According to one embodiment, two methods of IS analysis may be implemented at cell 500 of pad matrix 140 shown in **Figure 5**. One IS method is the Differential IS method, which measures the current through the reference electrode and the active electrode when the potential is induced through the common electrode. **Figure 7** illustrates one embodiment of a cell 500 configured for the Differential IS analysis method. In one embodiment, the switch configuration is such that switches S_0, S_2, and S_4 are off while switches S_1, S_3, S_5, S_6 and S_7 are on. Also, select bits S_8 and S_9 are controlled by the user to control the gain of amplifier 510.

[0054] In one embodiment, if both electrodes have the same electrical behavior and surface characteristics, the current is zero (e.g., no binding or activity occurs on electrode). However, any small impedance change between the active and the reference electrode (e.g., binding of specific molecules into the active electrode surface) causes a differential current to be observed. This technique enhances the performance by suppressing the effects of common impedances between the common electrode and both active and reference electrode.

[0055] The second analysis method is the Single-Ended IS method. In the Single-Ended IS method, the impedance of the electrode-electrolyte is obtained in

frequency ω by measuring the voltage amplitude and phase across a known load impedance in series with the unknown system impedance. **Figure 8** illustrates one embodiment of cell 500 configured for the Single-Ended IS analyses method with an external load impedance.

[0056] In one embodiment, the load impedance is configured as a tuned circuit (e.g., piezo-electric crystal) for higher sensitivity. In another embodiment, the load impedance is configured as the input impedance of the differential amplifier itself. The switch configuration for the Single-Ended IS method with external load impedance is such that switches S_0 and S_2 are off while switches S_1, S_3, S_4 and S_7 are on. Switches S_5 and S_6 are in a don't care state.

[0057] **Figure 9** illustrates one embodiment of cell 500 configured for the Single-Ended IS analyses method with no external load impedance. The switch configuration for the Single-Ended IS method with no external load impedance is such that switches S_0, S_2, and S_3 are off while switches S_1, S_4 and S_7 are on. Switches S_5 and S_6 are in a don't care state.

CV (Differential and Single Ended)

[0058] Cyclic Voltammetry (CV) analysis and other potential sweep methods are used to obtain the complete electrochemical behavior of a system. This is achieved by introducing a series of different potentials waveforms and steps, and recording the current-potential curves obtained (Fig.8). **Figure 12** illustrates one embodiment of an interactive test for CV analysis. Like the IS analyses method, CV analysis features Differential analysis and Single-Ended IS analyses.

[0059] According to one embodiment, the sensor cell 500 circuitry is unchanged from the IS analysis method discussed above (e.g., **Figures 7-9**). However in CV analysis, the electrode is driven to a condition far from equilibrium and the response (usually a transient signal from non-linear elements) is observed. The large potentials in multiple CV can be measured by the circuit topology. The Single-Ended and Differential CV methods have different applications, but overall the differential method has much more sensitivity when studying electrodes interfacial behavior.

CV (Differential and Single Ended)

[0060] The other feature of the SEBA 100 is its capability to be used as an Electrophoretic system. An Electrophoretic system is implemented to repel or attract specific ions. **Figure 10** illustrates one embodiment of cell 500 configured as an Electrophoretic system. In one embodiment, the switch configuration is such that switches S_3, S_4, S_5, S_6 and S_7 are off while switches S_0, S_1, and S_2 are on. In addition, select bits S_8 and S_9 are also off. In a further embodiment, all three electrodes are controlled by external voltage supplies. In yet another embodiment, Electrochemical synthesis is also possible with this topology.

[0061] Whereas many alterations and modifications of the present invention will no doubt become apparent to a person of ordinary skill in the art after having read the foregoing description, it is to be understood that any particular embodiment shown and described by way of illustration is in no way intended to be considered limiting. Therefore, references to details of various embodiments are not intended to limit the scope of the claims, which in themselves recite only those features regarded as essential to the invention.

[0062] Thus, a semiconductor electrochemical biosensor array has been described.